PhD Thesis

Vascular invasion detected by orcein staining and its significance in different tumours, with emphasis on colorectal cancer

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LIST OF FULL PAPERS THAT SERVED AS THE BASIS OF THE PHD THESIS

I. Sejben I., Bori R., Cserni G.
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II. Cserni G., Sejben I., Bori R.
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III. Sejben I., Kocsis L., Török L., Cserni G.
Elastic staining does not assist detection of venous invasion in cutaneous melanoma.
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1. INTRODUCTION

Every year, approximately 1.2 million new cases of colorectal cancer (CRC) are discovered and about 600,000 patients die of the disease all over the world. CRC is the second most common cause of cancer-related mortality in both sexes causing about 5,000 deaths per year in Hungary. Adenocarcinomas comprise more than 90% of CRC.

To improve CRC patient management, the prognosticators of these tumours should be more precisely determined. The College of American Pathologists Consensus Statement in 1999 stratified the prognostic parameters into five categories according to the published strength of evidence regarding their prognostic value. Blood and lymphatic vessel invasion (BLVI) has been established to be a category I prognostic factor in CRC along with local extent of tumour invasion, regional lymph node metastases, residual tumour, preoperative serum carcinoembryonal antigen level and radial margin involvement. BLVI has not only prognostic importance but may also influence therapy. Dukes B colon cancer patients are often recommended adjuvant chemotherapy on the basis of clinico-pathological factors, such as bowel obstruction, less than 12 lymph nodes examined, high grade, BLVI, perineural invasion, localized perforation and positive, close or undetermined resection margins.

Elastica stainings are reported to be useful in the evaluation of venous invasion (VI) in colorectal, gastric and oesophageal cancers. The use of elastic stains can provide a more precise identification of VI in CRC compared to routine haematoxylin and eosin (H&E) stain, and the demonstration of venous involvement by an elastic stain in colorectal carcinoma specimens is associated with a higher risk of distant metastasis. We undertook a two-phase study to investigate the prognostic significance of VI demonstrated by orcein stain in CRC. Along the same lines, we tried to visualize VI in sections from cutaneous melanoma specimens using orcein staining.

2. AIMS

2.1 To investigate the frequency of VI in CRC specimens detected on H&E and orcein-stained slides separately and to compare the results.

2.2 To assess VI found on H&E and orcein stain and its relation to distant metastasis in CRC.

2.3 To confirm the association between VI identified by orcein staining in CRC and the development of distant metastasis after an extended follow-up period.

2.4 To look for differences between cancers with VI that develop distant metastases and those without metastases despite venous involvement.

2.5 To determine the benefit of orcein elastic staining of primary cutaneous melanoma specimens in detecting VI.

3. MATERIALS AND METHODS

3.1 THE STUDY OF ORCEIN DETECTED VI AND ITS ASSOCIATION WITH DISTANT METASTASES IN CRC

In the first phase of our CRC study, CRC resection specimens received at the Department of Pathology in 2007 were retrospectively collected from an institutional database. Besides the classification into pT categories reflecting the depth of invasion or invasion of the peritoneum or nearby organs, and pN categories reflecting the nodal status, the initial M categories for the presence or absence of distant metastases were derived from data available at the multidisciplinary meetings. For the initial M categories, intraoperative biopsy and histology proven metastases (pM) and clinically detected metastases (M) were considered. For the initial staging, patients were evaluated routinely by chest radiograms and abdominal ultrasound, and further imaging studies were performed when required. During follow-up, the M category evaluation was also complemented by autopsy-derived data and patients' charts were used as source. All tumour blocks were prospectively and routinely assessed for VI in slides stained with orcein. First the H&E-stained slides were assessed for VI, and this was followed by the examination of the synchronously stained orcein slides. The results on VI were reported as detected on H&E or orcein. The tumours were therefore also categorised according to the V classification of the TNM: V1 for the presence of microscopically detected VI, and V0 for its absence. Follow-up data were available up to April 2009. The following cases were excluded from the analysis: intramucosal carcinomas with no invasion beyond the muscularis mucosae (pTis according to the TNM classification), recurrent cancers, cases treated with neoadjuvant therapies before histopathological evaluation, cases with synchronous or metachronous cancers elsewhere in the body, and MX

cases due to either lack of follow-up data or postoperative death without autopsy. The association of VI or nodal involvement and distant metastases was evaluated with the Fisher exact test. Significance level was set at $p<0.05$ (two tailed).

In the second phase of our study, the same series of tumours was reanalysed after a follow-up period extending to February 2014. Patients lost to follow-up were censored, and their metastasis status was recorded according to the last follow-up month. Parameters of cases demonstrating VI with (M1) an without (M0) distant non-peritoneal metastasis at the time of diagnosis or later during follow-up were compared with each other. The orcein stained slides of the CRCs in the compared subsets were reviewed with the number and localisation (intramural or extramural) of VI being recorded. VI density was determined by the overall number of invaded veins divided by the number of orcein stained slides (number of tumour blocks). Contingency tables were used for assessing the associations of nodal or VI status and distant metastasis; the Fisher exact test and the t-test for independent samples were used for the comparison of categorical and continuous variables, respectively. Significance level was set at $p<0.05$, and all tests were two tailed.

3.2 THE STUDY OF UTILITY OF ORCEIN STAINING TO DETECT VI IN CUTANEOUS MELANOMA

We conducted a second study to evaluate the usefulness of orcein stain to detect VI in cutaneous melanomas. Forty cases of primary cutaneous melanoma in vertical growth phase, surgically resected between January 2006 and September 2013 were retrieved from the institutional archives. Only cases with minimum tumour thickness of 3 mm were included. All tumours were at least Clark level III. All tumour blocks were stained originally with H&E and subsequently with orcein. Two pathologists simultaneously assessed the slides for vascular invasion at a multiheaded microscope. First the H&E-stained slides then those stained with orcein were examined. The results with the two staining methods were recorded separately and compared with the original reports.

4.RESULTS

3.1 THE STUDY OF ORCEIN DETECTED VI AND ITS ASSOCIATION WITH DISTANT METASTASES IN CRC

After the exclusion of the cases mentioned in Materials and Methods, 89 patients (47 men and 42 women) remained for analysis. These included 16 patients with rectal tumours and 73 with colon tumours. The median number of orcein-stained slides was 6. VI was detected in 16/89 (18%) cases on H&E-stained slides, whereas it was found to be present in 63/89 (71%) cases after the evaluation of orcein-stained slides. VI was more common with greater pT categories and was also more common with nodal involvement; all tumours classified as pN2 had VI. Eleven cases had distant metastases at the initial staging. The median number of lymph nodes examined was 18 (range 4-55). There were 46 (52%) node-positive cases, nine of the M1 tumours were among these, whereas two metastatic cases belonged to the node-negative patients. The lymph node status (pN0 versus node positive) nearly showed an association with an initial M1 status ($p=0.05$). VI detected by H&E was not associated with an initial M1 status ($p=1$), but VI detected by orcein stain showed a significant association with synchronously detected distant metastasis ($p=0.029$). Ten M0 cases with less than 6 months of follow-up after surgery were excluded from subsequent analyses. Of the remaining 79 tumours 14 (18%) demonstrated VI on H&E slides and this number rose to 56 (71%) after the analysis of orcein-stained slides. Chemotherapy was administered to 45 patients (30 with VI and nodal involvement, 11 with VI alone, three with nodal involvement alone, and one with no VI or nodal metastasis) and no systemic therapy was given to 34 patients (six with VI and nodal involvement, eight with VI alone, one with nodal involvement alone, and the remaining 19 with no VI or nodal metastasis). For the 68 cases that were initially M0, the median follow-up time was 17 months (range 6-28 months); during this period nine patients with colon cancer developed distant metastases after 4-22 months (median 9 months) following surgery. Five of the 20 finally metastatic cases were detected in the node-negative group, all the 20 metastatic cases had VI detected by the orcein stain. Synchronous or metachronous distant metastases were associated with lymph node involvement and VI detected by orcein ($p=0.02$ and $p=0.001$, respectively), whereas H&E detected VI showed no associations ($p=0.31$). VI detected on orcein stain predicted the presence of distant metastases with an accuracy of 0.54, a sensitivity of 1.0 and a specificity of 0.39. The accuracy, sensitivity and specificity for nodal metastases to foretell distant metastases were found to be 0.54, 0.75 and 0.56.

In the second phase of the CRC study, the median follow-up time for the repeated analysis was 48 months. On review of the original data and slides, some patients had their metastatic, VI and follow-up status changed and were therefore added, leaving 87 CRC patients (45 males and 42 females; median age 71 years, range 29-88 years; 16 with rectal and 71 with colon cancer) for further analysis of the association between metastatic disease and VI. Sixty-one patients (70 %; 95% confidence interval (CI): 59-79%) had VI identified with the orcein stain, 18(21%; CI: 13-31%) also had VI identified on H&E-stained slides. Fourty-six patients received chemotherapy (29 with both VI and nodal involvement, 12 with VI alone, 4 with nodal metastasis alone and 1 without VI or nodal disease) and 41 (19 with no VI or nodal involvement, 10 with only VI, 2 with only nodal disease and 10 with both nodal involvement and VI) did not. Thirty-one patients have died, 18 of or with disease and 13 of unrelated causes. In addition to the 10 patients initially staged as M1 on the basis of distant non-peritoneal metastasis, further 16 patients developed distant metastasis after a median of 24 months following surgery. Two metachronous metastatic cases (both node-positive and one positive for VI) had only peritoneal involvement. As these are unlikely to be explained by VI or nodal involvement, they were not considered as distant (haematogenous) metastasis for the purpose of the analyses, where only non-peritoneal distant metastases were taken into account. If nodal status and the presence of any VI detected by orcein were tests to predict the developement of distant spread (excluding peritoneal spread), on the basis of our data, the accuracy, sensitivity and specificity of these would be 59.8%, 70.8%, 55.6% and 52.9%, 91.7%, 38.1% respectively. The associations with distant metastasis of a node-positive status (FET, p=0.03) and of VI detected by orcein staining (FET, p=0.008) were significant. H&E detected VI showed no significant association with metastases (FET, p=0.24). Of the 61 cases with identified VI, 39 did not develop distant non-peritoneal metastasis (V1M0 group) and 22 had either synchronous (n=10) or metachronous (n=12) distant non-peritoneal metastasis (V1M1 group). Of the parameters analysed, the age, gender ratio, pT or pN categories, the number of lymph nodes examined or involved, the number of cases with H&E detected VI and the number of orcein stained slides showed no significant differences. The average follow-up period of patients in the V1M0 group was greater, whereas their mean VI density and the proportion of cases having extramural rather than solely intramural VI was lower than in the V1M1 group. Using a non-inclusive cut-off of 1 for VI density (i.e. at least one instance

of orcein-detected VI per slide on average considered significant; n=14 with this feature), the association with distant non-peritoneal metastasis was found to be significant (FET, p=0.024) and the accuracy, sensitivity and specificity were 70.5%, 40.9% and 87.2%, respectively. Considering only orcein-detected extramural VI, the association with distant non-peritoneal metastasis was significant, and the accuracy, sensitivity and specificity were 60.0%, 90.9% and 42.1%, respectively. Regarding the relation between the localisation of VI and its density, the mean (±S.D.) VI density was higher in cases with extramural VI (n=42) than in cases having only intramural VI (n=18) (1.05±0.98 versus 0.31±0.16; $p<0.0001$), and there were also more cases with VI detected on H&E slides in CRCs with extramural VI (17 versus 1, p=0.01). However, most cases with VI (n=37) had both intramural and extramural VI detected by the orcein stain, with two additional cases having extramural VI and VI of undeterminable localisation and one further case with intramural VI and VI of undeterminable localisation. The VI density of cases with intramural VI (n=56) and those only extramural VI (n=3) was not significantly different.

3.2 THE STUDY OF UTILITY OF ORCEIN STAINING TO DETECT VI IN CUTANEOUS MELANOMA

The depth of invasion of the melanomas studied ranged between 3.1 and 18 mm. On average, 4 tumour-containing blocks were examined from each case. Vascular invasion was detected in 10 of 40 cases on H&E-stained slides. Blood vessel and lymphatic involvement could not be differentiated on H&E-stained slides. Orcein stain highlights venous involvement. Definite VI was identified with orcein in 5 cases. All but one definite VI detected with orcein were recognised on H&E-stained slides too. Six cases of vascular invasion detected with H&E were not seen with orcein. There was only one case where orcein contributed to the identification of more veins involved, but part of these was revealed by H&E too. To sum up, orcein stain did not improve the detection rate of VI. Furthermore, the detection of VI was made difficult by remaining elastic fibres of actinic dermal elastosis, or the invasion of adnexal structures mimicking vascular invasion on orcein stain.

5. DISCUSSION

Mortality caused by CRC after potentially curative resection is most often related to local or distant recurrence. Spreading of cancer cells follows a variety of routes: direct spread, transperitoneal spread, implantation, lymphatic spread, haematogenous spread and venous extension. Haematogenous, lymphohaematogenous and peritoneal spreading are the three main pathways of cancer cell dissemination to distant sites. VI is a morphologically detectable crucial step in tumour progression and spreading, which explains its presence among CRC prognostic factors besides the depth of tumour invasion through the layers of the bowel wall, lymph node involvement and the presence of distant metastases. The presence of blood vessel invasion is an obvious prerequisite for the development of blood-borne metastases.

To detect VI, the following structures should be recognized in an ideal situation: lumen with tumour cell embolus and blood or thrombus, endothelial lining, circular smooth muscle cells and elastic fibres in vein wall, adjacent artery. Recognizing the luminal tumour cell and blood or thrombus rarely needs special stains. If swollen endothelial cells resemble neoplastic cells, cytokeratin and general endothelial markers can solve the issue. Endothelium, which is specific to vascular structures, can be highlighted by immunohistochemical markers. Factor VIII-related antigen, CD31 and CD34 are used to highlight lymphatic as well as blood vessel endothelial cells. On H&E-stained slides, no reliable distinction can be made between lymphatic and blood vessels. Immunohistochemical lymphatic endothelial markers can be applied to differenciate between them. However, it must be stressed that the endothelium of invaded veins is often destroyed beyond recognition. Histochemical or immunohistochemical stain for smooth mucle cells and elastic staining for elastin, which stain the smooth muscle and the elastin in the wall of veins respectively, can be used to enhance the detection of VI. Verhoeff's stain, Weigert's stain, Miller'stain and orcein are the most widely used staining methods to detect elastic fibres. Elastic stains also highlight the accompanying artery the presence of which aid the identification of veins.

The reported incidence of VI in CRC shows marked variability ranging from 10 to 90%. Assuming that in all patients with distant haematogenous metastases VI had been present, 10.5% and 29.6% false negative rates for VI were calculated by Ouchi and Sternberg, respectively. According to Sternberg et al, this variation is due to differences in the

characterictics of the tumours of the reported series, technical differences in specimen processing and interobserver variation. In other words, the detection rate of VI is influenced by tumour features (stage and differentiation), the amount of tumour examined (number and size of blocks and sections), staining methods (H&E versus elastic stain or immunostains) and the skill of the reporting pathologist. To minimize the false negative rate, they suggested increasing the number of blocks and slides, using elastic stains and cutting tangential blocks from the perimeter of the tumour, across the mesentery, and from mesenteric vessels. Their results indicate that there is a direct relationship between VI incidence and tumour stage, while inverse relationship was found between VI incidence and tumour differentiation, and the greater mean number of blocks was examined, the higher rates of VI were detected. According to the calculation of Talbot el al, 3.9% of cases with extramural VI would be missed if 5 blocks were taken, while 41.3% would be missed if only 2 blocks were processed. The Royal College of Pathologists recommends taking a minimum of 4 blocks to optimise the detection of key prognostic features. In the Consensus Statement of the College of American Pathologists, submitting of 5 or more tumour blocks is considered to be optimal.

VI has been defined by Talbot as „tumour present in endothelium-lined space surrounded by a rim of smooth muscle or containing red blood cells". This definition is used to identify venous involvement on H&E-staind slides, but does not help in identifying all instances of VI. Other signs have been implemented as markers of VI on H&E-stained slides, and these include the „protruding tongue" (smooth-bordered protrusion of tumour tissue into pericolorectal fat usually adjacent to an artery) and the „orphan artery" (a focus of circumscribed tumour with an adjoining artery, but no accompanying vein) signs.

Elastic stains have been implemented in the detection of VI, as they result in a higher visualisation. On average, a threefold increase in the VI detection rate was experienced in some studies comparing H&E and elastic stains. In this setting, tumour cells surrounded by an elastic lamina are considered VI. In their recent study, Kojima et al set uniform criteria to identify VI on elastic stained slides: "Presence of elastica-stained internal elastic membrane covering more than half of the circumference surrounding the tumour cluster".

The wide range of VI detection rates in CRC in different series indicates that the recognition of this phenomenon is not free of interobserver variability. Littleford et al

reported only poor-to-moderate agreement in the diagnosis of extramural VI on H&E-stained sections. Harris and colleagues investigated the impact of application of CD31 and D2-40 immunostains on interobserver variation on the reporting of VI and lymphatic invasion in stage II CRC. They found that interobserver agreement was poor irrespective of implementation of immunohistochemistry.

The rate of elastica stain detected VI being around 60-70% is higher than the proportion of CRC cases developing distant metastases. Therefore, VI has a false positive rate as a predictor of haematogenous metastases. Some phenomena called pseudo-VI predispose to overdiagnosing VI with special stains: arterial invasion, tangentionally sectioned subserosal elastic lamina, mucosal protrusion into the submucosa, periganglionic, perineural, perinodal and perifollicular elastic fibres or periglandular or perimuscular elastosis.

Veins can have a substantial thickening of their internal elastic lamina, and therefore, distinction from arterial invasion must be borne in mind. The presence of vascular structure and tumour cells or clusters within a circular structure may sometimes represent vascular wall invasion rather than real large vessel invasion with tumour cell in the lumen. These features can be mistaken for VI on H&E-stained slides. The identification of the internal elastic lamina surrounded by tumour cell clusters rather than the internal elastic lamina surrounding tumour cells may be a clue to identify these lesions as vascular wall invasion. The presence of a single endothelium-lined lumen in the central unaffected part of the lesion may further substantiate this. Some layers of the bowel wall may be bordered by elastic fibres, although these are not consistently present everywhere, and may range from virtually lacking to prominent, their prominence being possibly related to previous injury (e.g. neoadjuvant treatment). Their presence may sometimes take a circular form and therefore mimic the internal elastic lamina of a vein. Of these elastic fibre networks, the peritoneal, or subserosal elastic lamina, is probably the most important. It can be very prominent at some segments of the colon, and is particularly prone to form circular shapes in some planes of section. Elastic fibres may also occur beneath the muscularis mucosae, where diverticular protrusions of the mucosa into the submucosa may also give ground to pseudo-VI if such structures are involved by neoplastic glands. The presence of elastic fibre networks at specific anatomic/histologic layers should always be considered when dealing with elastica stain-detected vascular

invasion, although they represent differential diagnostic problems only when the tumour involves the tissues around them, and there are misleading planes of section.

The intermuscular elastic layer is generally not prominent, but can be accentuated around the ganglia of the myenteric plexus, which is not uncommonly involved by CRC infiltrating the muscularis propria layer.

The use of neoadjuvant radiotherapy or chemoradiotherapy in rectal cancer may lead to elastosis, the accumuation of elastic fibres at uncommon locations. In such conditions, periglandular fibres obviously create the situation of rounded structures that may mimic an internal elastic lamina. Muscle bundles may also be surrounded by elastic fibres in such situations, and this may also create a background for a pseudo-VI pattern if the tumour infiltrates these bundles.

Intraneural spread with perineural elastic fibres, very uncommonly perinodal elastic fibres (lymph node capsules are generally devoid of elastic fibres), an in anorectal carcinomas the perifollicular elastic sheet may give ground to the morphology of tumour cells surrounded by elastica positive layers, that is, pseudo-VI.

In conclusion, VI in CRC is believed to be a commonly underreported phenomenon. Taking at least 4-5 blocks, looking for orphan artery and protruding tongue signs, and application of an elastic stain on every tumour block are practical and easily introducible measures to reduce false negativity. However, the implementation of routine elastica staining increases the risk of false positivity. Therefore VI mimics should be excuded to achieve optimal results.

Although the frequency of VI is substantially increased by the use of an elastic stain, and this is of prognostic relevance, it is clear that not all tumours with identified VI develop distant metastasis. The sensitivity of VI for the development of synchronous or future metastases seemed idealistically perfect (100%) in our preliminary study with a rather short median follow-up of 17 months, but its specificity was much less optimal (39%). The association between the development of distant metastasis and orcein-detected VI (but not HE-detected VI) was significant. After a longer follow-up, this association was confirmed, and again HE-detected VI did not show a similar association. The sensitivity to identify

patients with distant metastasis any time in the course of their disease was still reasonably good (92%) after longer follow-up, its specificity remained low. From the clinical point of view, identifying a feature with higher specificity and positive predictive value would be ideal. In this respect lymph node involvement seems better, although its specificity of 56% is still not ideal. VI may be a logical source of non-peritoneal distant metastasis developing by haematogeneous spread, whereas lymph node metastasis may just be a reflection of a more advanced disease stage, although it may also lead to haematogenous spread on the longer term as lymph is finally drained to the venous system. This study tried to identify qualitative and/or quantitative features that may be different between patients who have VI but did not develop distant metastases and those who did.

Of the variables analysed, only three showed significant differences between patients in the V1M1 and V1M0 groups. The median follow-up of the first group was shorter, more people died in this group, in keeping with the fact that metastases confer a poor prognosis. If it had been the other way round, the follow-up of the V1M0 group being shorter, this would have reduced the credibility of the data. The longer period of follow-up of the second group also implies that these patients were really less likely to develop distant metastatic spread. Although there have been suggestions that both intramural and extramural VI have the same prognostic impact by entering the same vessels at different anatomic layers of the bowels, and we were of the same opinion, our findings suggest that extramural VI may carry a higher risk for metastasis than isolated intramural VI. The superiority of extramural VI over intramural VI as a prognostic factor is acknowledged by several guidelines, and our results are also in keeping with findings of previous and recent works in which both the presence of intramural VI and extramural VI had prognostic impact, but the latter had higher prognostic value.

Importantly, there seems to be a quantitative difference between patients with VI identified on orcein stained slides developing metastases during the course of their disease and those not developing them. This quantitative difference is reflected by the VI density in this study which was more than double on average in the M1 group as compared with the M0 group. Using an arbitrary cut-off of one VI per slide on average resulted in higher specificity for metastatic dissemination. Higher VI density values were also found to be associated with

greater metastatic incidence in two Japanese studies using elastica van Gieson staining to identify VI.

It seems obvious that one prognostic factor e.g. VI, however important it is, cannot predict the outcome of CRC. A combination of prognostic indicators is necessary to prognosticate more precisely and to determine patient management. For example, the Petersen prognostic index was created for stage II colon cancer patients, and is calculated on the basis of VI, peritoneal involvement, surgical margin involvement, and perforation through the tumour. A score of 1 was assigned to intramural or extramural VI, peritoneal involvement and margin involvement, whereas a score of 2 was allocated to tumour perforation. The total score was calculated by adding these scores. A total score of 0-1 was associated with a five year survival of 85.7% and defined the low risk group. Those having a total score of 2-5 belonged to the high risk group with a five year survival of 49.8%. This prognostic score has been validated in patients with Dukes B and C colon and rectal cancer too. In a collaborative study of our group, the performance of the Petersen index was compared in two centres. Although VI detection rate was significantly higher in one centre, and tumour perforation was more commonly detected in the other, the results confirmed the usefulness of this prognostic index. This also suggests that a single adverse parameter (unless it is transtumoural perforation considered with more weigh) has less effect on overall prognosis, and with that the tumour is still placed in the good prognostic category. This is in keeping with our present results, which also point to the fact that quantifying VI may make sense. A higher amount is associated with more frequent distant metastasis. Although the results are rather logical, the relatively low number of CRCs and patients in this cohort is a limitation that would require validation of the findings in a larger series.

As a conclusion, it may be reinforced that elastic stains increase the detection rate of VI in CRCs both in the context of frequency in patients with the disease and of quantity in patients with VI. Despite the association of orcein-detected VI and distant metastasis, the usefulness of orcein (or any other elastic stain) may be questioned as it identifies more patients with CRC and VI than the ones who will develop metastasis at a certain time of their disease, and advocating changes in therapy on this basis might result in overtreatment. By finding meaningful quantitative (VI density) and qualitative (only intramural versus

extramural VI) differences between VI-positive CRCs of patients with and without distant metastasis during follow-up, clinically meaningful VI could hopefully be better defined.

In cutaneous malignant melanoma, tumour thickness is the most important histopathologic prognostic factor. The studies on the significance of vascular invasion in melanoma came to different conclusions. Vascular invasion can be detected on standard slides, however, immunohistochemistry to demonstrate endothelial cells might be useful. The use of immunohistochemistry enhances the identification of vessel invasion in melanoma. To the best of our knowledge, there are no studies on the value of using elastic stains to identify vascular invasion in this tumour type, although these staining methods are reported to be useful in the evaluation of vascular invasion in colorectal, gastric and oesophageal cancer. The use of elastic stains can provide a more precise identification of vascular invasion in colorectal cancer compared to routine H&E stain. And the demonstration of venous involvement by an elastic stain in CRC specimens is associated with a higher risk of distant metastasis. Along the same lines, we tried to visualize VI in sections from cutaneous melanoma specimens using orcein staining. Five cases of definite VI were found with this method, while ten cases of vascular invasion were detected with H&E. That is, orcein did not augment the identification of vessel involvement. The cause of our results could be that melanomas invade lymphatic channels more frequently than blood vessels, and the blood vessels invaded are more often of the capillary type. Orcein stains the elastic fibres in the walls of relatively large veins and small blood and lymphatic vessels cannot be highlighted with it. Another reason lies within the histological structure of the skin containing a lot of elastic fibres in the dermis, which may sometimes be very disturbing as background, although some melanomas may lack this elastic debris, and push downward the elastotic layer associated with solar elastosis as they grow vertically. Elastic fibers are often arranged in circular lines, such as in periadnexal, perineural connective tissue, and can give rise to false positive results and make the distinction between VI and pseudo-VI difficult.

6. CONCLUSIONS

Our results suggest that VI is demonstrated with increased frequency in CRC with the orcein stain when compared with H&E staining. VI detected by orcein is associated with synchronous or metachronous distant metastasis similarly to lymph node metastasis.

Comparison of VI versus nodal status shows that although both are predictive for the development of distant metastases, the better sensitivity of the first is compensated by the better specificity of the other.

The localisation (intramural versus extramural) and the density/grade of VI demonstrated by orcein in CRC specimens determine its prognostic relevance. Although both intramural and extramural VI is associated with the development of distant metastases, the latter has greater prognostic value. A VI density of at least one VI per slide showed higher specificity for haematogenous dissemination.

On the basis of our investigations and the review of the literature, it is suggested that orcein or other elastica staining should be routinely used for the evaluation of VI in CRC, at least for the node-negative and H&E-based VI-negative cases.

The optimal detection of VI would therefore imply the evaluation of 4-5 tumour blocks, a search for morphological signs of VI (the “protruding tongue” and the “orphan artery” signs) on H&E stained slides, the routine use of an elastica staining, and a distinction between mimics of VI (pseudo-VI) and true VI.

Investigating into the applicability of elastic stain to detect VI in cutaneous melanoma, we conclude that orcein stain is not useful for improving the detection of vessel involvement in these tumours.

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